

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1-57. (Canceled).

58. (Currently Amended) A method for producing a nonadherent chicken embryonic stem cell line capable of proliferating in a basal medium in the absence of exogenous trophic factors and cytokines, said method comprising culturing chicken embryonic stem cells for at least 20 successive passages in a culture medium, wherein:

(a) in at least the first passage, the chicken embryonic stem cells are cultured in a primary medium comprising:

(i) ~~the following trophic factors: stem cell factor (SCF), factor~~ insulin-like growth factor 1 (IGF-1) ~~and basic fibroblast growth factor (bFGF); and the following cytokines: cytokine~~ ciliary neurotrophic factor (CNTF), ~~interleukin-6 (IL-6), soluble IL-6 receptor and interleukin-11 (IL-11);~~

(ii) an inactivated feeder comprising mouse fibroblast STO cells;
and

(iii) a basal medium selected from the group consisting of DMEM, GMEM, HamF12 and MacCoy medium, wherein the basal medium is supplemented with fetal calf serum at an initial concentration of from 8 to 12 %;

(b) the chicken embryonic stem cells obtained in step (a) are further cultured in a primary medium that has been modified by progressively depriving the primary medium of said trophic factors and said cytokines defined in (i) of step (a), wherein the progressive withdrawal of each trophic factor and cytokine is carried out by successive passage of the chicken embryonic stem cells in a culture medium having a lower concentration of at least one of said trophic factors or said cytokines as compared to the culture medium of the prior passage, until the medium is free of all exogenous trophic factors and cytokines; and

(c) the chicken embryonic stem cells obtained in step (b) are inoculated at a high density into a bacteriological dish to produce a nonadherent chicken embryonic stem cell line

capable of proliferating in a basal medium in the absence of exogenous trophic factors and cytokines.

59-60. (Canceled).

61. (Currently Amended) The method of claim 58, wherein the nonadherent chicken embryonic stem cell line obtained in step(c) is capable of proliferating in a basal medium in the absence of exogenous trophic factors and cytokines ~~cells derived from the lines obtained in step (e) are capable of proliferating~~ for at least 50 days.

62-65. (Canceled).

66. (Previously Presented) The method of claim 58, further comprising the following step (d):

(d) proliferating in suspension the nonadherent chicken embryonic stem cells derived from the lines obtained in step (c) in a medium free of exogenous trophic factors and cytokines.

67. (Canceled).

68. (Previously Presented) The method of claim 58, wherein the cells derived from the lines obtained in step (c) have a high nucleocytoplasmic ratio, an endogenous alkaline phosphatase activity, an endogenous telomerase activity, and a reactivity with specific antibodies selected from the group of antibodies SSEA-1, (TEC01) and EMA-1.

69. (Previously Presented) The method of claim 58, wherein the cells used in step (a) are obtained by suspending cells from blastodermal disks of fertilized eggs in a culture medium comprising the tropic factors and cytokines as defined in (i) of step (a), wherein said cells are inoculated into a feeder comprising mouse fibroblast STO cells, incubated, and then collected.

70. (Canceled).

71. (Previously Presented) The method of claim 58, further comprising the following step (d):

(d) proliferating cells derived from the lines obtained in step (c) in a basic medium selected from the group consisting of DMEM, GMEM, HamF12 and McCoy, wherein the medium is supplemented with various additives selected from the group consisting of nonessential amino acids, vitamins and sodium pyruvate.

72. (Canceled).

73. (New) The method of claim 58, wherein the primary medium of step (a) further comprises any one or more of the following trophic factors and cytokines: stem cell factor (SCF), basic fibroblast growth factor (bFGF), interleukin 6 (IL-6), soluble IL-6 receptor (sIL-6r) and interleukin 11 (IL-11).

74. (New) The method of claim 73, wherein the primary medium of step (a) further comprises the following trophic factors and cytokines: stem cell factor (SCF), basic fibroblast growth factor (bFGF), interleukin 6 (IL-6), and soluble IL-6 receptor (sIL-6r).

75. (New) The method of claim 73, wherein the primary medium of step (a) further comprises the following trophic factors and cytokines: stem cell factor (SCF) and interleukin 11 (IL-11).

76. (New) The method of claim 73, wherein the primary medium of step (a) further comprises the following trophic factors and cytokines: stem cell factor (SCF), basic fibroblast growth factor (bFGF), interleukin 6 (IL-6), soluble IL-6 receptor (sIL-6r) and interleukin 11 (IL-11).